PCT

(FORM PTO-1390 (Modified) (REV 11-2000)) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **1038-1226 MIS:jb**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): **10/088569**

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/CA00/01097	**September 21, 2000**	**September 22, 1999**

TITLE OF INVENTION

DNA IMMUNIZATION AGAINST CHLAMYDIA INFECTION

APPLICANT(S) FOR DO/EO/US

Robert C. Brunham

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (e) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)). – unsigned copy
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409)
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

    Initial Information Data Sheet

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
10/088569	PCT/CA00/01097	1038-1226 MIS:jb

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		CALCULATIONS PTO USE ONLY
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	$1040.00	
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	$890.00	
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	$740.00	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4)	$710.00	
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)	$100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =		$890.00
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).		$0.00

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	21 - 20 =	1	x $18.00	$18.00
Independent claims	4 - 3 =	1	x $84.00	$84.00
Multiple Dependent Claims **(check if applicable)**.			☐	$0.00
TOTAL OF ABOVE CALCULATIONS =				$992.00
☐ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				$0.00
SUBTOTAL =				$992.00
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	$0.00
TOTAL NATIONAL FEE =				$992.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				$0.00
TOTAL FEES ENCLOSED =				$992.00
			Amount to be: refunded	$
			charged	$

a. ☒ A check in the amount of **$992.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **192253** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Mr. Michael I. Stewart
Sim & McBurney
6th Floor, 330 University Avenue
Toronto, Ontairo
Canada, M5G 1R7.

24223
PATENT ,TRADEMARK OFFICE

Michael I. Stewart
SIGNATURE

Michael I. Stewart
NAME

24,973
REGISTRATION NUMBER

March 20, 2002
DATE

PTO/SB/17 (11-01)
Approved for use through 10/31/2002 OMB 0651-0032
Patent and Trademark Office U.S. DEPARTMENT OF COMMERCE

FEE TRANSMITTAL for FY 2002

Patent fees are subject to annual revision.

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT | **$130.00**

Complete if Known	
Application Number	10/088,569
Filing Date	
First Named Inventor	Robert C. Brunham
Examiner Name	
Group Art Unit	
Attorney Docket No.	1038-1226 MIS

METHOD OF PAYMENT *(check all that apply)*

☒ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

☐ Deposit Account

Deposit Account Number:

Deposit Account Name:

The Commissioner is authorized to: *(check all that apply)*

☐ Charge fee(s) indicated below ☐ Credit any overpayments

☐ Charge any additonal fee(s) during the pendency of this application

☐ Charge fee(s) indicated below, **except for the filing fee** to the above identified deposit account

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	740	201	370	Utility filing fee	
106	330	206	165	Design filing fee	
107	510	207	255	Plant filing fee	
108	740	208	370	Reissue filing fee	
114	160	214	80	Provisional filing fee	
				SUBTOTAL (1)	

2. EXTRA CLAIM FEES FOR UTILITY AND

	Extra Claims		Fee from below	Fee Paid
Total Claims		-20** = 0	X	= 0.00
Independent Claims		- 3** = 0	X	= 0.00
Multiple Dependent				=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	84	202	42	Independent claims in excess of 3
104	280	204	140	Multiple dependent claim, if not paid
109	84	209	42	** Reissue independent claims over original patent
110	18	210	9	** Reissue claims in excess of 20 and over original patent
				SUBTOTAL (2) $0.00

** or number previously paid, if greater, For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	130.00
127	50	227	25	Surcharge - late provisional filing fee or cover sheet	
139	130	139	130	Non - English specification	
147	2,520	147	2,520	For filing a request for *ex parte* reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	400	216	200	Extension for reply within second month	
117	920	217	460	Extension for reply within third month	
118	1,440	218	720	Extension for reply within fourth month	
128	1,960	228	980	Extension for reply within fifth month	
119	320	219	160	Notice of Appeal	
120	320	220	160	Filing a brief in support of an appeal	
121	280	221	140	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,280	241	640	Petition to revive - unintentional	
142	1,280	242	640	Utility issue fee (or reissue)	
143	460	243	230	Design issue fee	
144	620	244	310	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Processing fee under 37 CFR § 1.17(q)	
126	180	126	180	Submission of Information Disclosure Statement	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	740	246	370	Filing a submission after final rejection (37 CFR § 1.129(a))	
149	740	249	370	For each additional invention to be examined (37 CFR § 1.129(b))	
179	740	279	370	Request for Continued Examination (RCE)	
169	900	169	900	Request for expedited examination of a design application	
Other fee (specify)					

*Reduced by Basic Filing Fee Paid SUBTOTAL (3) $130.00

SUBMITTED BY				Complete *(if applicable)*	
Name *(Print/Type)*	Michael I. Stewart	Registration No. *(Attorney/Agent)*	24,973	Telephone	(416) 595-1155
Signature				Date	August 12, 2002

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Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re National Phase of International

Appl'n. No.	:	PCT/CA00/01097
Filed	:	September 21, 2000
Applicant	:	Robert C. Brunham
Title	:	DNA IMMUNIZATION AGAINST CHLAMYDIA INFECTION
Docket No.	:	1038-1226 MIS:jb

March 20, 2002

BY COURIER

The Commissioner of Patents
and Trademarks,
Washington, D.C. 20231,
U.S.A.

PRELIMINARY MENDMENT

Sir:

Please amend the above-identified application as follows:

In the Specification:

Before the first line of the specification, add the following:

" REFERENCE TO RELATED APPLICATIONS

This application is a national phase application under 35 U.S.C. 371 of PCT/CA00/01097."

REMARKS/ARGUMENTS

The specification has been amended on page 1 to reflect that this application is a U.S. National Phase filing under 35 U.S.C. 371 of PCT/CA00/01097.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "**Version with markings to show changes made**."

Respectfully submitted,
SIM & McBURNEY

Michael I. Stewart
Reg. No. 24,973

Toronto, Ontario, Canada,
(416) 595-1155
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Appl. No.

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

Before the first line of the specification, add the following:

" REFERENCE TO RELATED APPLICATIONS

This application is a national phase application under 35 U.S.C. 371 of PCT/CA00/01097."

TITLE OF INVENTION

DNA IMMUNIZATION AGAINST *CHLAMYDIA* INFECTION

FIELD OF INVENTION

The present invention relates to immunology and, in particular, to immunization of hosts using nucleic acid to provide protection against infection by *Chlamydia*.

BACKGROUND OF THE INVENTION

DNA immunization is an approach for generating protective immunity against infectious diseases (ref. 1 - throughout this application, various references are cited in parentheses to describe more fully the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification, immediately preceding the claims. The disclosure of these references are hereby incorporated by reference into the present disclosure). Unlike protein or peptide based subunit vaccines, DNA immunization provides protective immunity through expression of foreign proteins by host cells, thus allowing the presentation of antigen to the immune system in a manner more analogous to that which occurs during infection with viruses or intracellular pathogens (ref. 2). Although considerable interest has been generated by this technique, successful immunity has been most consistently induced by DNA immunization for viral diseases (ref. 3). Results have been more variable with non-viral pathogens which may reflect differences in the nature of the pathogens, in the immunizing antigens chosen, and in the routes of immunization (ref. 4). Further development of DNA vaccination will depend on elucidating the underlying immunological mechanisms and broadening its application to other infectious diseases for which existing strategies of vaccine development have failed.

Chlamydia trachomatis is an obligate intracellular bacterial pathogen which usually remains localized to mucosal epithelial surfaces of the human host. Chlamydiae are dimorphic bacteria with an extracellular spore-like transmission cell termed the elementary body (EB) and an intracellular replicative cell termed the reticulate body (ref. 5). From a public health perspective, chlamydial infections are of great importance because they are significant causes of infertility, blindness and

are a prevalent co-factor facilitating the transmission of human immunodeficiency virus type 1 (ref. 6). Protective immunity to *C. trachomatis* is effected through cytokines released by Th1-like CD 4 lymphocyte responses and by local antibody in mucosal secretions and is believed to be primarily directed to the major outer membrane protein (MOMP), which is quantitatively the dominant surface protein on the chlamydial bacterial cell and has a molecular mass of about 40 kDa (ref. 16).

Initial efforts in developing a chlamydial vaccine were based on parenteral immunization with the whole bacterial cell. Although this approach met with success in human trials, it was limited because protection was short-lived, partial and vaccination may exacerbate disease during subsequent infection episodes possibly due to pathological reactions to certain chlamydial antigens (ref. 8). More recent attempts at chlamydial vaccine design have been based on a subunit design using MOMP protein or peptides. These subunit vaccines have also generally failed, perhaps because the immunogens do not induce protective cellular and humoral immune responses recalled by native epitopes on the organism (ref. 9).

In copending US Patent Application No. 08/893,381 filed July 11, 1997, assigned to University of Manitoba and the disclosure of which is incorporated herein by reference (WO 98/02546), I have described the generation of a protective immune response using a DNA sequence which encodes the MOMP of *C. trachomatis* in a plasmid by DNA immunization.

SUMMARY OF THE INVENTION

The present invention is concerned with nucleic acid immunization, specifically DNA immunization, to generate in a host protective antibodies to a serine-threonine kinase of a strain of *Chlamydia*. DNA immunization induces a broad spectrum of immune responses including Th1-like CD4 responses and mucosal immunity.

Accordingly, in one aspect, the present invention provides a non-replicating vector comprising a nucleotide sequence encoding a serine-threonine kinase (STK) or a fragment of STK that generates a STK-specific immune response, and a promoter sequence operatively coupled to said nucleotide sequence for expression of said STK in a host to which the vector is administered.

The promoter may be a cytomegalovirus promoter, and may be contained in the human cytomegalovirus major immediate-early promoter-enhancer region. The vector may be a plasmid vector and the nucleotide sequence may be that of SEQ ID No: 1.

The strain of *Chlamydia* may be a strain of *Chlamydia* inducing chlamydial infection of the lung, including *Chlamydia trachomatis* or *Chlamydia pneumoniae*. The non-replicating vector may be plasmid pcDNA3 into which the nucleotide sequence is inserted. The pcDNA3 vector may contain the nucleotide sequence having SEQ ID No: 1.

In a further aspect of the present invention, there is provided an immunogenic composition for *in vivo* administration to a host for the generation in the host of a protective immune response to a serine-threonine kinase (STK) of a strain of *Chlamydia*, comprising a non-replicating vector as provided herein and a pharmaceutically-acceptable carrier therefor.

In an additional aspect of the invention, there is provided a method of immunizing a host against disease caused by infection with a strain of *Chlamydia*, which comprises administering to said host an effective amount of a non-replicating vector as provided herein.

In these aspects of the present invention, the various options and alternatives discussed above for the non-replicating vector may be employed.

The non-replicating vector may be administered to the host, including a human host, in any convenient manner, such as intramuscularly or intranasally.

The present invention also includes, in a further aspect thereof, a method of using a gene encoding a serine-threonine kinase (STK) of a strain of *Chlamydia* or a fragment of said STK that generates a STK-specific immune response, to produce an immune response in a host, which comprises isolating said gene; operatively linking said gene to at least one control sequence to produce a non-replicating vector, said control sequence directing expression of said STK or fragment thereof when introduced into a host to produce an immune response to said STK or fragment thereof; and introducing said vector into a host.

In an additional aspect of the invention, there is provided a method of producing a vaccine for protection of a host against disease caused by infection with a strain of *Chlamydia*, which comprises isolating a nucleotide sequence encoding a serine-threonine kinase (STK) of a strain of *Chlamydia* or a fragment of the STK that generates a STK-specific immune response, operatively linking said nucleotide sequence to at least one control sequence to produce a non-replicating vector, the control sequence directing expression of said STK or fragment thereof when introduced to a host to produce an immune response to said STK or fragment thereof, and formulating said vector as a vaccine for *in vivo* administration to a host.

The various options and alternatives discussed above may be employed in this aspect of the invention.

Advantages of the present invention, therefore, include a method of obtaining a protective immune response to infection carried by a strain of *Chlamydia* by DNA immunization of DNA encoding the major outer membrane protein of a strain of *Chlamydia*.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1A and Figure 1B show the results of immunization with serine-threonine kinase gene (pSTK) resulting in enhanced clearance of mouse pneumonitis (MoPn) infection in lung. Groups Balb/c mice were immunized with pSTK (n=5), pcDNA3 (n=6), saline (n=5) or with 1000 IFU of live MoPn EB (n=6). Fourteen days after last immunization, mice were challenged intranasally with infectious MoPn (2000 IFU). Figure 1A: body weight of the mice was measured daily after challenge infection until mice were sacrificed at day 10. Figure 1B: mice were sacrificed at postinfection day 10, and MoPn growth in lung was analyzed by quantitative tissue culture. Data are mean $\pm$ SE of $\log_{10}$ IFU/lung. *$p<0.05$, $p<0.01$ vs. pcDNA-treated group. Legend: EB=host-killed elementary bodies, STK=plasmid DNA, N=native, pcDNA3=empty vector.

Figure 2 shows the construction of plasmid pcDNA3/STK.

Figure 3 shows the nucleic acid sequence of the STK gene (SEQ ID No: 1).

GENERAL DESCRIPTION OF THE INVENTION

To illustrate the present invention, plasmid DNA was constructed containing the serine-threonine kinase (STK) gene from the *C. trachomatis* mouse pneumonitis strain (MoPn), which is a natural murine pathogen, permitting experimentation to be effected in mice. It is known that primary infection in the mouse model induces strong protective immunity to reinfection. For human immunization, a human pathogen strain is used.

Any convenient plasmid vector may be used, such as pcDNA3, a eukaryotic II-selectable expression vector (Invitrogen, San Diego, CA, USA), containing a human cytomegalovirus major-immediate-early promoter-enhancer region. The STK gene may be inserted in the vector in any convenient manner. The gene may be amplified from *Chlamydia trachomatic* genomic DNA by PCR using suitable primers and the PCR product cloned into the vector. The STK gene-carrying plasmid may be transferred, such as by electroporation, into *E. coli* for replication therein. Plasmids may be extracted from the *E. coli* in any convenient manner.

The plasmid containing the STK gene may be administered in any convenient manner to the host, such as intramuscularly or intranasally, in conjunction with a pharmaceutically-acceptable carrier.

The data presented herein and described in detail below demonstrates that DNA immunization with the *C. trachomatis* STK gene elicits immune responses and produces significant protective immunity to lung challenge infection with *C. trachomatis* MoPn.

It is clearly apparent to one skilled in the art, that the various embodiments of the present invention have many applications in the fields of vaccination, diagnosis and treatment of chlamydial infections. A further non-limiting discussion of such uses is further presented below.

1. Vaccine Preparation and Use

Immunogenic compositions, suitable to be used as vaccines, may be prepared from the STK genes and vectors as disclosed herein. The vaccine elicits an immune response in a subject which includes the production of anti-STK antibodies. Immunogenic compositions, including vaccines, containing the nucleic acid may be

prepared as injectables, in physiologically-acceptable liquid solutions or emulsions for polynucleotide administration. The nucleic acid may be associated with liposomes, such as lecithin liposomes or other liposomes known in the art, as a nucleic acid liposome (for example, as described in WO 9324640) or the nucleic acid may be associated with an adjuvant, as described in more detail below. Liposomes comprising cationic lipids interact spontaneously and rapidly with polyanions, such as DNA and RNA, resulting in liposome/nucleic acid complexes that capture up to 100% of the polynucleotide. In addition, the polycationic complexes fuse with cell membranes, resulting in an intracellular delivery of polynucleotide that bypasses the degradative enzymes of the lysosomal compartment. Published PCT application WO 94/27435 describes compositions for genetic immunization comprising cationic lipids and polynucleotides. Agents which assist in the cellular uptake of nucleic acid, such as calcium ions, viral proteins and other transfection facilitating agents, may advantageously be used.

Polynucleotide immunogenic preparations may also be formulated as microcapsules, including biodegradable time-release particles. Thus, U.S. Patent 5,151,264 describes a particulate carrier of a phospholipid/glycolipid/polysaccharide nature that has been termed Bio Vecteurs Supra Moléculaires (BVSM). The particulate carriers are intended to transport a variety of molecules having biological activity in one of the layers thereof.

U.S. Patent 5,075,109 describes encapsulation of the antigens trinitrophenylated keyhole limpet hemocyanin and staphylococcal enterotoxin B in 50:50 poly (DL-lactideco-glycolide). Other polymers for encapsulation are suggested, such as poly(glycolide), poly(DL-lactide-co- glycolide), copolyoxalates, polycaprolactone, poly(lactide-co-caprolactone), poly(esteramides), polyorthoesters and poly(8-hydroxybutyric acid), and polyanhydrides.

Published PCT application WO 91/06282 describes a delivery vehicle comprising a plurality of bioadhesive microspheres and antigens. The microspheres being of starch, gelatin, dextran, collagen or albumin. This delivery vehicle is particularly intended for the uptake of vaccine across the nasal mucosa. The delivery vehicle may additionally contain an absorption enhancer.

The STK gene containing non-replicating vectors may be mixed with pharmaceutically acceptable excipients which are compatible therewith. Such excipients may include, water, saline, dextrose, glycerol, ethanol, and combinations thereof. The immunogenic compositions and vaccines may further contain auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, or adjuvants to enhance the effectiveness thereof. Immunogenic compositions and vaccines may be administered parenterally, by injection subcutaneously, intravenously, intradermally or intramuscularly, possibly following pretreatment of the injection site with a local anesthetic. Alternatively, the immunogenic compositions formed according to the present invention, may be formulated and delivered in a manner to evoke an immune response at mucosal surfaces. Thus, the immunogenic composition may be administered to mucosal surfaces by, for example, the nasal or oral (intragastric) routes. Alternatively, other modes of administration including suppositories and oral formulations may be desirable. For suppositories, binders and carriers may include, for example, polyalkylene glycols or triglycerides. Oral formulations may include normally employed incipients, such as, for example, pharmaceutical grades of saccharine, cellulose and magnesium carbonate.

The immunogenic preparations and vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective, protective and immunogenic. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the individual's immune system to synthesize the STK and antibodies thereto, and if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgement of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art and may be of the order of about 1 g to about 1 mg of the STK gene-containing vectors. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations. The dosage may also depend on the route of administration and will vary according to the size of the host. A vaccine which protects against only one pathogen is a monovalent vaccine. Vaccines which

contain antigenic material of several pathogens are combined vaccines and also belong to the present invention. Such combined vaccines contain, for example, material from various pathogens or from various strains of the same pathogen, or from combinations of various pathogens.

Immunogenicity can be significantly improved if the vectors are co-administered with adjuvants, commonly used as 0.05 to 0.1 percent solution in phosphate-buffered saline. Adjuvants enhance the immunogenicity of an antigen but are not necessarily immunogenic themselves. Adjuvants may act by retaining the antigen locally near the site of administration to produce a depot effect facilitating a slow, sustained release of antigen to cells of the immune system. Adjuvants can also attract cells of the immune system to an antigen depot and stimulate such cells to elicit immune responses.

Immunostimulatory agents or adjuvants have been used for many years to improve the host immune responses to, for example, vaccines. Thus, adjuvants have been identified that enhance the immune response to antigens. Some of these adjuvants are toxic, however, and can cause undesirable side-effects, making them unsuitable for use in humans and many animals. Indeed, only aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as adjuvants in human and veterinary vaccines.

A wide range of extrinsic adjuvants and other immunomodulating material can provoke potent immune responses to antigens. These include saponins complexed to membrane protein antigens to produce immune stimulating complexes (ISCOMS), pluronic polymers with mineral oil, killed mycobacteria in mineral oil, Freund's complete adjuvant, bacterial products, such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well as monophoryl lipid A, QS 21 and polyphosphazene.

In particular embodiments of the present invention, the non-replicating vector comprising a first nucleotide sequence encoding a STK gene of *Chlamydia* may be delivered in conjunction with a targeting molecule to target the vector to selected cells including cells of the immune system.

The non-replicating vector may be delivered to the host by a variety of procedures, for example, Tang et al. (ref. 14) disclosed that introduction of gold microprojectiles coated with DNA encoding bovine growth hormone (BGH) into the skin of mice resulted in production of anti-BGH antibodies in the mice, while Furth et al. (ref. 15) showed that a jet injector could be used to transfect skin, muscle, fat and mammary tissues of living animals.

2. Immunoassays

The STK genes and vectors of the present invention are useful as immunogens for the generation of anti-STK antibodies for use in immunoassays, including enzyme-linked immunosorbent assays (ELISA), RIAs and other non-enzyme linked antibody binding assays or procedures known in the art. In ELISA assays, the non-replicating vector first is administered to a host to generate antibodies specific to the STK. These STK specific antibodies are immobilized onto a selected surface, for example, a surface capable of binding the antibodies, such as the wells of a polystyrene microtiter plate. After washing to remove incompletely adsorbed antibodies, a nonspecific protein, such as a solution of bovine serum albumin (BSA) that is known to be antigenically neutral with regard to the test sample, may be bound to the selected surface. This allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific bindings of antisera onto the surface.

The immobilizing surface is then contacted with a sample, such as clinical or biological materials, to be tested in a manner conducive to immune complex (antigen/antibody) formation. This procedure may include diluting the sample with diluents, such as solutions of BSA, bovine gamma globulin (BGG) and/or phosphate buffered saline (PBS)/Tween. The sample is then allowed to incubate for from about 2 to 4 hours, at temperatures such as of the order of about 20 to 37 C. Following incubation, the sample-contacted surface is washed to remove non-immunocomplexed material. The washing procedure may include washing with a solution, such as PBS/Tween or a borate buffer. Following formation of specific immunocomplexes between the test sample and the bound STK specific antibodies,

and subsequent washing, the occurrence, and even amount, of immunocomplex formation may be determined.

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.

Example 1:

This Example shows the preparation of a plasmid vector for immunization.

The *C. trachomatis* mouse pneumonitis (MoPn) isolate was grown in HeLa 229 cells in Eagle MEM containing 10% fetal bovine serum and 2 mM L-glutamine. The MoPn EBs were harvested and purified by step gradient density centrifugation at 43,000g for 60 min at 4°C. The purified EBs were washed twice with PBS, centifugated at 30,000g for 30 min, resuspended in sucrose-phosphate-glutamic acid (SPG) buffer and frozen at -70°C until used.

The serine-threonine kinase (STK) gene was cloned into eukaryotic expression plasmid, pcDNA3 (Invitrogen, San Diego) to form plasmid pcDNA3/STK. The STK gene was amplified from MoPn genomic DNA by polymerase chain reaction (PCR) with a 5' primer (GGG GAT CCG CCA CCA TGC TTG AAT TAG GCG TAT CGT TTC CT - SEQ ID No: 2) which included a *Bam*HI site, a start codon, and the N-terminal sequence of the mature serine-threonine kinase of MoPn and a 3' primer (GGG GCT CGA GCT ATT ACC GGA CTC TTT TTA AGC TGA TAA G - SEQ ID No: 3) which include a *Xho*I site, two stop codons (CTA TTA), and the C-terminal sequence of the *MoPn* STK gene. After digestion with *Bam*HI and *Xho*I, the PCR product, having the sequence shown in Figure 3 (SEQ ID No: 1), was cloned into *Bam*HI and *Xho*I restricted pcDNA3 with transcription under the control of human cytomegalovirus major intermediate-early promoter-enhancer region. The STK gene-encoding plasmid was transferred by

electroporation into *Escherichia coli* DH5α, which was grown in Luria-Bertani (LB) broth containing 100 μg/ml ampicillin. The plasmid was extracted by a DNA purification system (Wizard Plus Maxiprep; Promega, Madison, WI), and the sequence of recombinant STK DNA was verified by PCR direct sequence analysis. Purified plasmid DNA was dissolved in saline at a concentration of 1 mg/ml. The DNA concentration was determined by spectrophotometry (DU-62; Beckman, Fullerton, CA) at 260 nm, and the size of the plasmid was compared with DNA standards in a ethidium bromide-stained agarose gel.

Example 2:

This Example shows the results of immunizing studies using the plasmid vector.

Female Balb/c mice (4 to 5 weeks old) were purchased from Charles River Canada (St. Constant, Canada) mice were intramuscularly and intranasally immunized with plasmid DNA, prepared as described in Example 1, on three occasions, at 0, 2 and 4 weeks. For each immunization, a total of 200 μg DNA in 200 μl was injected into the two quadriceps muscles (100 μg of DNA/injection site) using a 27-gauge needle. At the same time, 50 μg DNA in 50 μl was delivered onto the nostrils of mice with a micropipette. The droplet was subsequently inhaled by the mice.

Mice were challenged intranasally with $2x10^3$ IFU of *C. trachomatis* MoPn EB 14 days after last immunization, as described. Briefly, after ether anesthesia 25 μl of SPG containing an inoculum of $2x10^3$ IFU of MoPn was delivered onto the nostrils of mice with a micropipette. The droplet was subsequently inhalted by the mice. Body weight was measured daily for 10 days following the challenge infection as a measure of chlamydia-induced morbidity. On postinfection day 10, the mice were sacrificed and their lungs were aseptically isolated and homogenized with grinder in SPG buffer. The tissue suspensions were centrifuged at 500g for 10 min at 4°C remove coarse tissue and debris. Supernatants were frozen at -70°C until tissue culture testing for quantitative growth of the organism.

For more direct measure of the effectiveness of the DNA vaccination, the ability to limit the *in vivo* growth of *Chlamydia* following a sublethal lung infection

was evaluated. In this infection model system, postchallenge day 10 is the time of peak growth and was chosen for comparison of lung titers among the various groups of mice. Mice immunized with STK DNA had a lung titer (log_{10} IFU) is 31.6 and 316.2 folds lower than negative control groups (blank vector and saline groups).

SUMMARY OF DISCLOSURE

In summary of this disclosure, the present invention provides a method of nucleic acid, including DNA, immunization of a host, including humans, against disease caused by infection by strain of *Chlamydia*, specifically *C. trachomatis*, employing a non-replicating vector, specifically a plasmid vector, containing a nucleotide sequence encoding a serine-threonine kinase (STK) of a strain of *Chlamydia* and a promoter to effect expression of STK in the host. Modifications are possible within the scope of this invention.

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CLAIMS

What I claim is:

1. A non-replicating vector comprising:

a nucleotide sequence encoding a serine-threonine kinase (STK) or a fragment of said STK that generates a STK-specific immune response, and

a promoter sequence operatively coupled to said nucleotide sequence for expression of said STK in a host to which the vector is administered.

2. The vector of claim 1 wherein said promoter sequence is a cytomegalovirus promoter.

3. The vector of claim 2 wherein the cytomegalovirus promoter is contained in the human cytomegalovirus major immediate-early promoter-enhancer region.

4. The vector of claim 1 which is a plasmid vector.

5. The vector of claim 1 wherein said nucleotide sequence has SEQ ID No: 1.

6. The vector of claim 1 wherein said strain of *Chlamydia* is a strain producing chlamydial infections of the lung.

7. The vector of claim 1 wherein said strain of *Chlamydia* is a strain of *Chlamydia trachomatis*.

8. The vector of claim 7 wherein said non-replicating vector comprises plasmid pcDNA3 containing said promoter sequence and into which said nucleotide sequence is inserted in operative relation to said promoter sequence.

9. The vector of claim 8 wherein said nucleotide sequence has SEQ ID No: 1.

10. An immunogenic composition for *in vivo* administration to a host for the generation in the host of a protective immune response to a serine-thresnine kinase (STK) of a strain of *Chlamydia*, comprising a non-replicating vector as claimed in claim 1, and a pharmaceutically-acceptable carrier therefor.

11. A method of immunizing a host against disease caused by infection with a strain of *Chlamydia*, which comprises administering to said host an effective amount of a non-replicating vector as claimed in claim 1.

12. A method of using a gene encoding a serine-threonine kinase (STK) of a strain of *Chlamydia* or a fragment of said STK that generates a STK-specific immune response, to produce an immune response in a host, which comprises:

isolating said gene,

operatively linking said gene to at least one control sequence to produce a non-replicating vector, said control sequence directing expression of said STK or fragment thereof when introduced into a host to produce an immune response to said STK or fragment thereof, and

introducing said vector into a host.

13. The method of claim 12 wherein said control sequence is a cytomegalovirus promoter.

14. The method of claim 13 wherein the cytomegalovirus promoter is contained in the human cytomegalovirus major immediate-early promoter-enhancer region.

15. The method of claim 12 wherein said non-replicating vector is a plasmid vector.

16. The method of claim 12 wherein said nucleotide sequence has SEQ ID No: 1.

17. The method of claim 12 wherein said strain of *Chlamydia* is a strain producing chlamydial infections of the lung.

18. The method of claim 12 wherein said strain of *Chlamydia* is a strain of *Chlamydia trachomatis*.

19. The method of claim 12 wherein said non-replicating vector comprises plasmid pcDNA3 containing said control sequence into which said gene encoding STK is inserted in operative relation to said control sequence.

20. The method of claim 19 wherein said nucleotide sequence has SEQ ID No: 1.

21. The method of claim 12 wherein said host is a human host.

22. A method of producing a vaccine for protection of a host against disease caused by infection with a strain of *Chlamydia*, which comprises:

isolating a nucleotide sequence encoding a serine-threonine kinase (STK) of a strain of *Chlamydia* or a fragment of said STK that generates a STK-specific immune response,

operatively linking said nucleotide sequence to at least one control sequence to produce a non-replicating vector, the control sequence directing expression of said STK or fragment thereof when introduced to a host to produce an immune response to said STK or fragment thereof, and

formulating said vector as a vaccine for *in vivo* administration to a host.

23. A vaccine produced by a method as claimed in claim 22.

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(54) Title: DNA IMMUNIZATION AGAINST *CHLAMYDIA* INFECTION

(57) Abstract: Nucleic acid, including DNA, immunization is used to generate a protective immune response in a host, including humans, to a serine-threonine kinase (STK) of a strain of *Chlamydia*. A non-replicating vector, including a plasmid vector, contains a nucleotide sequence encoding an STK or a fragment of the STK that generates antibodies that specifically react with STK and a promoter sequence operatively coupled to the first nucleotide sequence for expression of the STK in the host. The non-replicating vector may be formulated with a pharmaceutically-acceptable carrier for *in vivo* administration to the host.

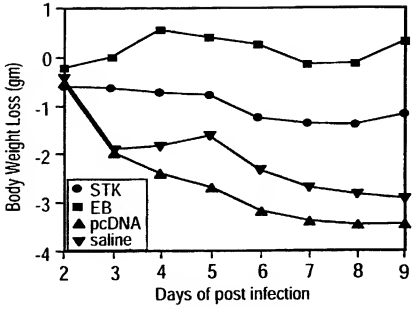
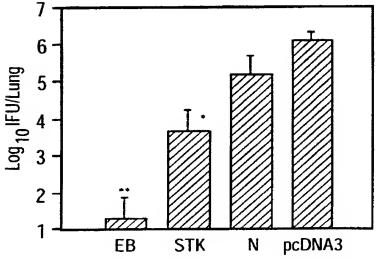


FIG.1A



* p<0.05, ** p<0.01, when compared with pcDNA3 group

FIG.1B

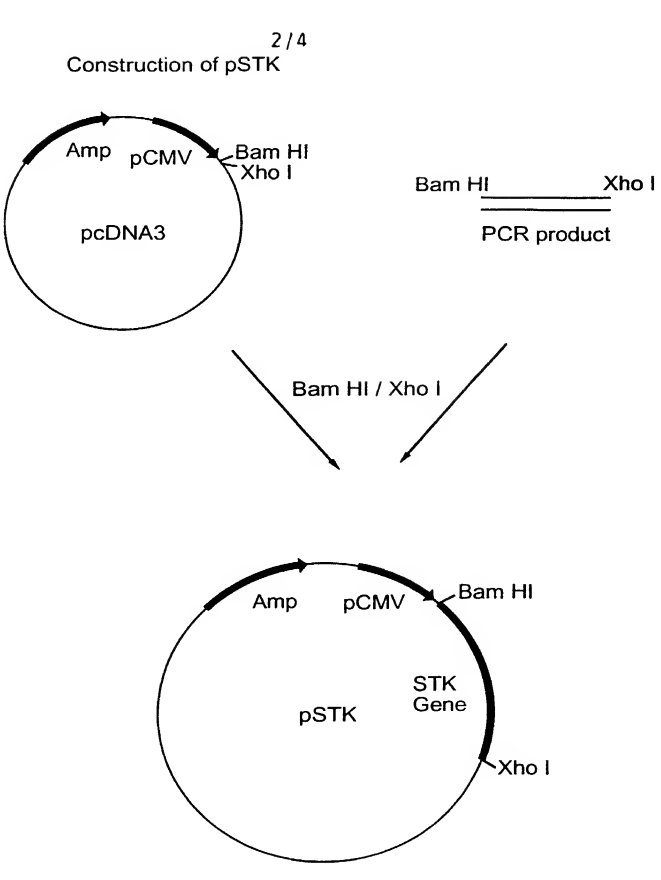

Construction of pSTK
Amp
pCMV
Bam HI
Xho I
pcDNA3
Bam HI
Xho I
PCR product
Bam HI / Xho I
Amp
pCMV
Bam HI
STK
Gene
pSTK
Xho I

FIG.2

10/088569
PCT/CA00/01097

FIG.3A

Chlamydia trachomatis Serine threonine kinase gene (STK)

>stk gene, 1467 bases

1 ATG CTT GAA TTA GGC GTA TCG TTT CCT TCC AAG ACT AAA TAT CTT 45
46 CTG ACA CGA GAA CTT AGT CGT AAG GTA GGC TTG ACT GTC TAT CAA 90
91 GGA GTG GAT GAG AGT TCT TCT CGT CCT GTG GTG ATC AAA GCA TTG 135
136 GTA TCT CCA GGG ATT CAT GAC CAG CGT TTT CTT CGT GCT TTT GAA 180
181 GAA GAA GCT AGG ATT ATG CAA CTT GTA GAT CAT CCG GCA TTT GTT 225
226 CGA TTA GAA GAA AAA GGT GAG TGG GAG CAA GGA CGT TAT TTC GTT 270
271 TCT GAA TAT ATT TTA GGG CAT TCA TTG CGA GAT ATT ATC CTT TCA 315
316 TCT CAT CTC GCT TTG GAT AAG GCA GTT TCT ATT GTT TTA CAA GTA 360
361 GCG CAG GCA ATA ACG GCT CTT CAT AAA CAT CAT GTT TTA CAT CTC 405
406 GAT ATT AAA CCT GAA AAC ATC ATG ATT TCT CGG TTG GGA GAG GTC 450
451 AAG TTG ATC GAT TAT GGG CTT TCA GCC TGG CAA TTT AAT CAT TGG 495
496 GGT TCG CCT GCA TAT ATG AGT CCC GAA CAG AGC AGG CAG GAA AAG 540
541 CTA TCT CCC GCA TCC GAT GTG TAT GCT TTA GCT TTG TTA GCT TAT 585
586 GAG CTG ATT ATG GGG CAG CTT TCT TTA GGA AAG GTC TAT TTA TCT 630
631 TTA CTC CCC GTA AAG ATT AGT AAA GTG TTA ACT CAA GCA TTG CAA 675
676 CCA GAC CCA GAA GCA CGG TTT CCT TCT ATG CAA GAG TTT GCT ACG 720
721 GCT TTG CAA GAT TAT CTT ATG CAT GAT GTG CAC GAA GAT TAT CGT 765
766 AAA AAA GAT CGC GTA ATC ATG CAG TTT GAA CAG TTG CAG CAA CAA 810
811 AAT ATG TGG CTG GCT CCA GAT AAG CTT TGC ATG CCG GAA GGG ATG 855
856 GCT CTG CAC ATT TAT TCA CAA AAA GAG CCC TGT GAT TTA CAT AAT 900
901 GTT TAC TAT GAT ATA CTT AGG TCT GAG GAT ATA GTA GAA TTG TGG 945

FIG.3B

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946   TTC TGT TAT GCT CAG GGG CAC TGT AGT TTT GCT CTT AGT ATG ATC   990
991   AAA CAG TTT CTT AAT CAG CGA ACA GAG AAA GCG CAA GAT ATC CCA  1035
1036  ACA GTA ATA AAA ACA TTG GAT ACT CTT TGT AAA ACA ATG CAT ATT  1080
1081  CCG CTT TGT GAA AAA GGG ATT TCC TGC TGC TGT TTT ATA TTT TTC  1125
1126  CAA CAA GAA CTC ATG TGC TTT TCT TGT GGG AAA ACT GAT TTC TCG  1170
1171  TTA AAA AAG CAA ACG AGG GGA GTG CAA CGT TTT CAA GCG GAA TCG  1215
1216  CAA GGA ATA GGG GAA GAG GGA CCC CTG GAG ATC CAC AAA CAA TCT  1260
1261  TTT TTG TGG GAA CCT GGT GAT GAG CTT ATC GTA CAC ACC CCG AGG  1305
1306  GCT AGA GAT TTG GTA TAT TTA TAC TGT CCT TCT TTC CTG AAG TTG  1350
1351  CAA GAT AGA GGG CAA ATG GAT ATA TTC TGC CAA ACA GAT TAC CTT  1395
1396  CAG AAG GAA GTG AGG CAG AAG TAT GAC GGA AGT CTT TAT CCT TCA  1440
1441  ACA CTT ATC AGC TTA AAA AGA GTC CGG                          1467
```

Docket No.
1038-1226 MIS

Declaration and Power of Attorney For Patent Application

English Language Declaration

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

DNA IMMUNIZATION AGAINST CHLAMYDIA INFECTION

the specification of which
(check one)

☐ is attached hereto.

☒ was filed on Septemer 21, 2000 as United States Application No. or PCT International Application Number PCT/CA00/01097

and was amended on _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)			Priority Not Claimed
(Number)	(Country)	(Day/Month/Year Filed)	☐
(Number)	(Country)	(Day/Month/Year Filed)	☐
(Number)	(Country)	(Day/Month/Year Filed)	☐

I hereby claim the benefit under 35 U.S.C. Section 119(e) of any United States provisional application(s) listed below:

(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U. S. C. Section 120 of any United States application(s), or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, C. F. R., Section 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

PCT/CA00/01097	September 21, 2000	
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
09/401,780	September 22, 1999	Pending
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)
(Application Serial No.)	(Filing Date)	(Status) (patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. *(list name and registration number)*

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